RESIDUAL EFFECTS OF PESTICIDES ON PARASITISM BY *APHYTIS HOLOXANTHUS* DEBACH (HYMENOPTERA: APHELINIDAE) ON FLORIDA RED SCALE *CHRYSOMPHALUS AONIDUM* (L.) (HOMOPTERA: DIASPIDIDAE) IN CITRUS GROVES OF FLORIDA.

BY

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Abstract of Dissertation Presented to the Graduate School
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RESIDUAL EFFECTS OF PESTICIDES ON PARASITISM BY *APHYTIS HOLOXANTHUS* DEBACH (HYMENOPTERA: APHELINIDAE) ON FLORIDA RED SCALE CHRYSOMPHALUS AONIDUM (L.) (HOMOPTERA: DIASPIDIDAE) IN CITRUS GROVES OF FLORIDA.

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Aphytis holoxanthus Debach is an important biological control agent of Florida red scale. Laboratory and field tests were conducted to quantify the mortality of this hymenopterous parasite caused by field residues of carbaryl and dicofol. Behavior and mortality of adult *Aphytis holoxanthus* on carbaryl treated 'Hamlin' orange leaves were related to quantities of dislodgeable pesticidal residues over time as field weathering of residues progressed. Our data showed that field weathered carbaryl residues killed A. holoxanthus for a period of up to 22 d post-treatment under spring conditions in Florida. Dicofol residues, on the other hand, were less toxic to the adult parasites. In another laboratory study, the rates of contact of *A.*

holoxanthus with different carbaryl deposit patterns varied significantly (F = 13.16; df = 11; P < 0.0001). The rate of contact was highest on leaf discs containing 20 deposits with and without carbaryl, while leaf discs having 2 large deposits of the same total volume (20 μl) resulted in lower contact rates. The field study which was designed to understand the disruption of the host-parasite relationship by carbaryl and dicofol spray applications revealed that the mean survival rate of 3rd instar nymphs and gravid females on leaf samples was higher in the carbaryl treatment than in the dicofol and control treatments due to lower rates of parasitism. There was no significant difference in survival of 3rd instar nymphs and gravid females between the dicofol and control treatments. In September, the parasitism of FRS was significantly higher in the control and dicofol treatments than in the carbaryl treatment. Fruit infestation likewise was highest in the carbaryl treatment followed by the dicofol and control treatments. The likelihood ratio chi square test showed significantly lower fruit infestation and fruit drop in the control treatment than in the carbaryl and dicofol treatments.

CHAPTER 1
INTRODUCTION

The Florida red scale (FRS), *Chrysomphalus aonidum* (L.) (Homoptera: Diaspididae), was considered the most important pest of citrus in Florida, Texas, Israel and other areas during the 1950s. Subsequently, this serious pest was brought under complete biological control in most areas by the introduction of an effective ectoparasite, *Aphytis holoxanthus* Debach from Hong Kong in 1956 (Hymenoptera: Aphelinidae)(Rosen, 1967; Clancy et al. 1963; Rosen and Debach, 1978).

In Florida, citrus is an important horticulture crop and its successful commercial production is more or less dependent on pesticidal applications to control key arthropod pests and diseases such as scales, mites, fungi etc. Chemical control of citrus pests has always been quite expensive due to the large size of citrus trees (Rosen, 1974). It is conservatively estimated that in Florida total spray material and application cost on citrus was $186 million during 1994 (Muraro and Hebb, 1994a; Muraro et al., 1994b; Muraro et al. 1994c). According to Bedford and Cillers (1987) highly toxic pesticide sprays in South Africa caused upsets of minor non-target pests so that one spray led to another until some growers were spraying up to 5 times, some as many as 9 times per season. The cost of chemical control may become prohibitive, with the ever-rising prices of modern pesticides. Chemical control is not always effective, i.e, certain citrus pests (e.g scales, mealybugs etc.) being rather difficult to control with chemicals. Pesticide-induced outbreaks of coccid and mite pests have been abundantly recorded on citrus in all major producing countries (Rosen, 1974). The rapid increase of armored scale populations in conjunction with repeated remedial applications of broad-spectrum synthetic pesticides often results in serious disruption of host-parasite relationships (Bartlett, 1953). Debach and Bartlett (1951) demonstrated the virtual impossibility of maintaining effective populations of *Aphytis* species in the continuous presence of synthetic organic insecticides, whether applied to control scale insects or for other citrus pests. Such pesticides also may constitute serious hazards to both the grower and consumer. With public concern over pesticidal pollution, increased demand for pesticide-free fruit is expected. Biological control, on the other hand, has been more successful in citriculture than in any other agricultural crop (Rosen, 1974).

With the problems of chemical control on the one hand, and the great potential for biological control on the other hand, the development of integrated control programs on citrus is desirable and is feasible in most citrus-growing countries (Rosen,1974). Integrated approaches to pest management complementary to both biological and chemical tactics often can be devised if an assessment has been made of the preferential toxicities of pesticides to pest species and natural enemies. At present in Florida, there is very little knowledge about toxicities of different commercial pesticides to these natural enemies. As the basis for an ecological approach to citrus pest control problems, the goal of this study was to characterize disruption caused by pesticides on selected host-parasite relationships as representative of biological control in the citrus ecosystem. More knowledge of the comparative effects of different pesticides on beneficial organisms would help in minimizing the effects of pesticide applications on biological control while effectively managing an existing pest problem. To attain this goal the objectives of the studies reported here were as follows:

1. To quantify the *A. holoxanthus* mortality induced by field-weathered residues of carbaryl and dicofol.
2. To investigate the carbaryl and dicofol residual degradation time by quantifying the field-weathered dislodgeable residues and to relate these residues to *A. holoxanthus* mortality.

3. To observe the behavior of *A. holoxanthus* to different deposit patterns of pesticides and their effects on mortality.
4. To characterize the pesticidal disruption of the host-parasite relationship.

This study is presented in 6 chapters. After a brief history of pesticidal treatments on citrus and their impact on scale and other insects (Chapter 2), the third chapter presents mortality results from the laboratory bioassay and degradation curves for dislodgeable pesticidal residues on field-weathered "Hamlin" orange leaves. Chapter 4 describes the effects of pesticidal deposit patterns on behavior and mortality of *A. holoxanthus*. Chapter 5 includes the details of field inoculation of Florida red scale and release of *A. holoxanthus*, documentation of the host-parasite relationship and details of the pesticidal disturbance and its effects on FRS population density and parasitism by *A. holoxanthus*. The concluding chapter summarizes the effects of pesticidal treatments on *A. holoxanthus* mortality, behavior and on host-parasite relationships, and addresses the implication of these results for integrated citrus pest management.

CHAPTER 2
LITERATURE REVIEW

Citrus is reported to have been propagated in Florida since colonization of St. Augustine in 1565, having been introduced from the old world through commerce. Similarly, the major complex of arthropod pests on citrus arose through introduction of exotic species with early movement of potted citrus plants into Florida. The relative status of the arthropod pests and disease organisms affecting citrus has changed over time. Emergence of new management technology such as the development of synthetic pesticides has dramatically altered the pest complex, reducing the pest status of some species while elevating that of others. Within this changing citrus ecosystem, pesticides also have modified the complexes of natural enemies existing in groves and thus have indirectly changed species pest status (Browning, 1994). Woglum et al. (1947) divided the period of 1907-1947 into three distinct pesticide use eras in California: the period of intermittent treatment prior to the availability of white oil sprays; the period of white oil sprays; and the period of the new synthetic organic compounds. At the beginning of the final period, fumigation was the only method of application in general use and a very small proportion of California citrus orchards were treated annually. This was a period when scales were the primary orchard pests, with citrus mealybug, *Plannococcus citri* (Risso)(Homoptera: Pseudococcidae), and red spider mite, *Panonychus citri*(McGregor)(Acari: Tetranychidae), causing occasional damage. *Aphytis chrysomphali* (Mercet) (Hymenoptera: Aphelinidae), formerly abundant in every red scale infested grove, persisted during this era but was usually abundant only in heavily infested trees which received limited pesticidal treatments or were in semi-abandoned condition.

Periodic colonizations of *Aphytis chrysomphali* and *Aphytis* "*A*" in 1949 by Debach et al. (1950) resulted in a satisfactory degree of commercial control of the California red scale (CRS), *Aonidiella aurantii* (Mask.)(Homoptera: Diaspididae), in several orchards. Trends in parasitization, scale density, developmental stages of scale, and proportions of dead and stung scale were used to indicate the need for supplemental parasite colonizations.

Armored scale insects (Homoptera: Diaspididae) constitute one of the most important groups of pests in agriculture. Many species are especially destructive to fruit trees and ornamentals, and their chemical control is often very difficult. Ectoparasites are by far the most effective, most promising natural enemies of armored scale insects. They are confined to the aphelinid genus *Aphytis* Howard. All the known species of *Aphytis* develop ectoparasitically on the body of armored scale insects beneath the scale covering. Introduced as well as naturally occurring species of *Aphytis* are the most important natural enemies of the Florida red scale, California red scale, purple scale, *Lepidosaphes beckii* (Newman) and many other injurious pests (Rosen, 1973).

The species recognition of *Aphytis holoxanthus* Debach, a parasite of Florida red scale, was concealed for many years by the common assumption that it was *A. lingnanensis* Comp (Homoptera: Aphelinidae). Extensive laboratory mating tests utilizing pure cultures of various species or collections in reciprocal crossing attempts demonstrated that *A. holoxanthus* would not cross with *A. lingnanensis* and other *Aphytis* species (Debach, 1960). During 1956-57, the aphelinid parasites *Aphytis holoxanthus* and *Pteroptrix smithi* (Compere) were introduced into Israel in an attempt to control FRS on citrus. Since the mid-1960s, *Chrysomphalus aonidum* is effectively controlled by these parasites coexisting in the citrus groves of Israel (Steinberg et al., 1986). In the fall of 1960, *A. holoxanthus* Debach was introduced from Hong Kong and Israel into central Florida. The parasite soon became established and in 1 year dispersed to most of the citrus growing areas of state. Rapid establishment, increase, and natural spread indicated that this parasite was well adapted to Florida conditions (Clancy et al., 1963; Selhime et al., 1969; Browning, 1994).

According to Bartlett (1953) the increase in populations of citrus pests formerly held in favorable biological balance has been an important effect of treatment with many of the newer, persistent-residue contact insecticides. Another general effect of pesticides has been exceptionally rapid flare-back of treated pests following initial control by broad spectrum pesticides. Both of these phenomena are consequences of the chemical destruction of natural enemies. The first type of disturbance usually results in the application of still another material; the second, by shortening the holding period between treatments, commonly results in more frequent applications of the same material that initiated the upset (Bartlett, 1953). Debach and Bartlett (1951) demonstrated the virtual impossibility of maintaining effective populations of *Aphytis* in California citrus in the continuous presence of synthetic organic insecticides, whether applied to control red scale or for other citrus pests. Abdel-Rahman (1973) concluded that integration of malathion and biological control of red scale in Australia did not seem possible.

Biological control of the cottony-cushion scale, *Icerya perchasi* Mask.,(Homoptera: Margarodidae), by the Vedalia beetle *Rodolia cardinalis* (Muls.)(Coleoptera: Coccinellidae)in desert citrus areas of California was upset so severely during 1956-1957 by insecticidal drift from treatment of surrounding areas that chemical control of the scale became necessary. *Rodolia* disruption was most often caused by drift of malathion, parathion, or DDT applied by airplane during the early spring months (Bartlett and Lagace, 1960). Similarly, California red scale tended to increase more rapidly on fruit in the parathion-sprayed orchards of South Africa right up to the picking season (Bedford, 1968). A severe drop of heavily scale-infested leaves and fruit was observed in the treated plots as early as August. Similarly, fruit drop was highest in DDT treated plots in Israel (Ben-Dov and Rosen, 1969).

The organophosphate insecticides azinphos-methyl and parathion, the insect growth regulator hydroprene and the miticide cyhexatin were highly toxic to either juvenile or adult *A. melinus* Debach and their field applications were viewed as potentially detrimental on, or adjacent to, citrus where scale is under biological control by *Aphytis* species (Davies and McLaren, 1977). A laboratory study by Cohen et al. (1988) showed that immature stages of *Aphytis* *holoxanthus* were unaffected by a malathion bait mixture (13% malathion) applied to their host, *Chrysomphalus aonidum*. However, emerging adults of *Aphytis holoxanthus* were highly susceptible and it was shown that malathion absorbed into the scale cover of the host was responsible for the lethal effect.

Peleg (1983) demonstrated that 0.1% fenoxycarb, an insect growth regulator, did not affect the normal development of the immature stages of parasites when applied as a spray to FRS parasitized by *Aphytis holoxanthus*. In a similar study, Peleg (1987) showed that the development and adult emergence of treated larvae and pupae of *Aphytis holoxanthus* parasitizing scales of FRS on squash were not affected by 9 ppm avermectin B1 (abamectin) treatment, but the fecundity of emerging females was markedly reduced. Citrus leaves sprayed with this concentration had some residual toxicity to adults of *Aphytis holoxanthus* for at least one day after treatment.

Bartlett (1963) tested the contact toxicity of sixty-one pesticidal residues in the laboratory against five hymenopterous parasites and six predatory coccinellids. Bartlett's data suggest that the effects of each pesticide upon most adult parasitic hymenoptera may be anticipated with a high degree of reliability, while the effect upon predatory coccinellids is much less predictable. According to Dean (1982), pesticides often were associated with mortality of *A. holoxanthus* when Florida red scale became an economic pest. The most pronounced increase in FRS populations in Texas occurred during July - September, although an occasional early increase was observed in May. *Aphytis holoxanthus* populations varied considerably, but parasite increase was usually found during the latter four months of the year. The most apparent increase in FRS populations and associated decrease in *Aphytis* populations followed the use of certain pesticides. Numerous instances were observed where a rapid increase of this scale insect followed the grower's repeated use of carbaryl (Dean, 1982).

Two sprays of abate (temephos) or delnav (dioxathion) for the control of citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae), on citrus trees were shown to cause heavy infestations of California red scale in South African orchards under integrated control (Bedford, 1971). A study on citrus thrips conducted by Bellows et al. (1985), concluded that in general, acephate had the longest residual effect among materials tested on beneficial organisms, followed by dimethoate, formetanate hydrochloride, and sabadilla. Mortality caused by acephate was closely related to the amount of leaf surface residues found in the chemical analysis. The same was true for dimethoate with *Aphytis melinus* and *Euseius stipulatus* (Mesostigmata, Phytoseiidae).

Formetanate hydrochloride caused high mortality of *Aphytis melinus* shortly after the application but inexplicably declined, even though the quantity of residue found by chemical analysis remained essentially unchanged. Sabadilla had almost no impact on any of the three natural enemies used in the experiment. The insecticides were compared with regard to the length of time the residues affected the beneficial organisms and showed that in the case of *Aphytis melinus*, acephate residue caused 50 percent mortality 23 days after treatment, whereas, residues of dimethoate and formetanate hydrochloride had ceased to cause 50 percent mortality after day 8. Bellows et al. (1985) recommended that after dimethoate has been used, a 10 day post application period would be required before releases of *Aphytis melinus* might result in establishment. A similar period would be required after use of formetanate hydrochloride, a longer period (23 days) after acephate, and no post-application release delays following application of sabadilla.

A carbaryl-resistant strain of the California red scale parasite, *Aphytis melinus*, was shown to be cross resistant to several citrus pesticides and could be utilized effectively in a citrus integrated pest management (IPM) program where pesticidal applications limit natural enemies (Spollen and Hoy,1993). The other pesticides included in the resistance analyses were supracide (methidathion), lorsban (chlorpyrifos), cygon (dimethoate) and carzol (chlordimeform).

Javier et al. (1991) studied field-collected populations of *Aphytis holoxanthus* and *Aphytis lignanensis* that were selected for malathion and azinphos-methyl resistance, respectively, and showed that fertility of virgin females was not significantly lower than that of mated females. Rates of development of their progeny also were similar. Males proved more susceptible to pesticides than females, and among them the sons of virgins were more susceptible than the sons of mated females. Resistance to malathion in *Aphytis holoxanthus* did not increase through 25 male selection cycles. Resistance of *Aphytis lignanensis* to azinphos-methyl, which had already been improved by mass selection, was further increased by 7 cycles of male selection. Havron et al. (1991) discovered high levels of azinphos-methyl resistance in a field population of *Aphytis lignanensis* parasitizing California red scale in a heavily sprayed Israeli citron orchard. Initial levels of permethrin tolerance were about 1.9 times higher than those in a susceptible population, indicating cross-resistance.

Alexandrakis (1990) found that the ectoparasite *Aphytis chilensis* Howard always had mortality rates higher in areas sprayed by air than in those sprayed by ground and the differences were greater during the summer months. The overall average rates of parasitism were 5 times higher in the ground sprayed areas than those sprayed by air. However, the total rates of parasitism in the areas sprayed by ground, which also took into consideration the dead *Aphytis chilensis*, did not differ substantially from rates calculated for the areas which were covered with aerial sprays. In general, *Aphytis chilensis* had a high mortality during late summer in the sprayed areas. Browning (1994) stated that the improvements in utilization of existing natural enemies are dependent upon detailed ecological and biological investigations to unravel the role that these important organisms play in natural control of insect pests.

CHAPTER 3
RESIDUAL EFFECTS OF CARBARYL AND DICOFOL ON *APHYTIS HOLOXANTHUS* DEBACH(HYMENOPTERA: APHELINIDAE).

Introduction

Prior to 1962, Florida red scale (FRS), *Chrysomphalus aonidum* (L.) (Homoptera: Diaspididae), was one of the most serious pests of Florida citrus (Clancy et al., 1963). Introduced into Florida from Cuba in 1874 (Mathis, 1947), it remained a serious problem for grapefruit growers until the parasite, *Aphytis holoxanthus* Debach, (Hymenoptera: Aphelinidae) was introduced in 1960 from Israel (Browning,1994). This parasitic wasp quickly reduced FRS to the status of an infrequent pest, and thereafter scale populations only increased periodically following the misuse of chemical pesticides. At present in Florida, there is very little knowledge about toxicities of different commercial pesticides to *A. holoxanthus*. The rapid increase of armored scale populations in conjunction with repeated remedial applications of broad-spectrum synthetic pesticides can, and often does, result in serious disruption of host-parasite relationships (Bartlett, 1953). This study was designed to quantify the mortality of *A. holoxanthus* induced by carbaryl and dicofol field residues and to relate this mortality to quantified field-weathered surface residues of the test materials.

Materials and Methods

Florida red scale rearing: Butternut squash (*Cucurbita maxima* Duchesne) fruit were used as a factitious host for Florida red scale due to their long shelf life and ease of handling (Rose, 1990). Squash were thoroughly washed in water, rinsed in a 5% sodium hypochlorite solution for about 3 minutes, rinsed in tap water and air dried. The uninfested host fruit were then inoculated with first instar nymphs (crawlers) from squash previously infested by FRS. Florida red scale culture rooms were maintained at 24±1°C, about 60% relative humidity and were physically isolated from the *A. holoxanthus* rearing room.

Parasite rearing: The rearing of *A. holoxanthus* involved parasitization of appropriately-aged host scales for 24 to 48 h. Squash with parasitized host scale then were transferred into a holding cage which contained no adult wasps and subsequently were transferred to an emergence cage after 12-13 d. This cage was used to provide adult *Aphytis* for culture cycling and for experimentation. A constant supply of honey was available on a piece of tissue paper rolled against the wall of emergence cages. Water was supplied with a plastic petri dish with a cotton wick through the cover.

Field pesticide application: The field experimental design was a randomized complete block with three treatments and four replications. Treatments consisting of Sevin wettable powder (Sevin 80S: Rhone-Poulenc, Alexander Drive, Research Triangle Park, NC 27709) at 567 g carbaryl per 378 liters of water (1.25 lb of carbaryl per 100 gal) and dicofol (Kelthane MF: Rohm & Haas, Philadelphia, PA 19105) at 473 ml per 378 liters of water (1 pint of dicofol per 100 gal) were applied with a commercial air blast sprayer to 'Hamlin' orange trees on Swingle citrumelo rootstock located at the Citrus Research and Education Center, University of Florida, Lake Alfred, on 20 March, 1995. Grove size was 2 hectares and the average tree height of the 10-year-old trees was 3 m. Treated plots were 2 rows with 2 trees in each, totaling 4 treated trees per replicate. Each plot was buffered by at least 3 rows of trees and 8 trees along the row. Four randomly selected control plots were left unsprayed. The minimum air temperature during the 31 d test period varied from 6.1 - 19.4°C (±1°C) and the maximum temperature fluctuation was 18.9 - 32.8°C (±1°C). The average minimum temperature for 31 d of this experiment was 14.8 ±1°C and average maximum temperature was 28.2 ±1 °C. The total rainfall during this period was 3.80 ±1 cm (1.21

cm on day 13, 1.88 cm on day 18, and 0.71 cm on day 21).

Mortality: Four mature, hardened leaves per tree were picked randomly from the outer 15 cm periphery of treated trees for bioassay of residual toxicity to adults of *Aphytis holoxanthus*. Adult parasites were assayed in this experiment because this is the mobile stage and thus most at risk to exposure to leaf surface residues. *Aphytis* eggs and larvae develop beneath the scale covering of host FRS and are less at risk to direct exposure.

Leaves from each treatment were collected into prelabelled paper bags enclosed in a plastic bag. A 4.5 cm disc was punched from each field-collected leaf and was placed into a ventilated plastic petri dish of 4.9 cm diameter in the laboratory.

Honey was streaked on the petri dish cover as a food source. Live adult *A. holoxanthus* were collected on every bioassay day from emergence cages into small tapered tube aspirators (7.6 cm long). A ventilation system was designed to deliver a gentle stream of humid air through each bioassay unit to minimize fumigation and avoid desiccation of leaf tissues and parasites. The ventilation system included an in-line charcoal, glass-wool filter. Air was bubbled through a flask of water for humidification. The filtered, humified air passed into the capillary tubes through a 3/4" PVC pipe manifold. The air flow from each capillary tube of the ventilation system was equilibrated and calibrated to 80-90 cc/min with an air flow meter (Dwyer instruments, IND 46360). Treatments were randomly assigned to locations on the ventilation system prior to collection of field samples to keep the treatment locations "blind" to observers. Each plastic tube contained 20 adult parasites less than 24-h-old and was attached to a capillary tube of the bioassay ventilation system (Fig. 3.1). Assessment of parasite mortality due to field-weathered leaf residues occurred at post-treatment intervals of 4 h, 1, 2, 3, 5, 7, 10, 14, 18, 22, 27 and 31 d, at which time parasite mortality on carbaryl treated leaves declined to the level of the untreated control. Each day, new, clean petri dishes and 20 adult *A. holoxanthus* $\leq$ 24-h-old were bioassayed. The bioassay was conducted under laboratory conditions of 24±2°C, and about 60%R.H. Each bioassay replicate had four observations and there were four replicates per treatment per day. Mortality data were collected from each post-treatment residue period after 24 h exposure of adult parasites by detaching the petri dishes from the ventilation system and counting the live and dead adult *Aphytis* in each petri dish. Adult *Aphytis* were anesthetized with a gentle flow of CO_2 through the petri dish before counting to reduce their activity. Petri dishes were then stored at -4°C for later assessment of sex ratios.

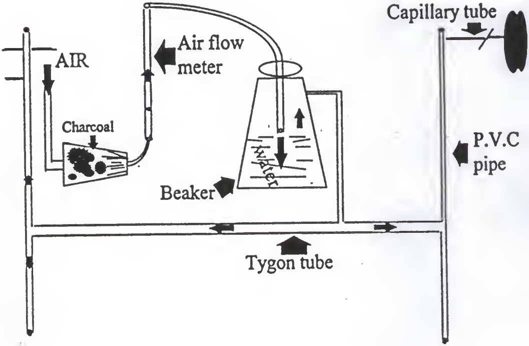

Figure 3.1. Ventilation system of bioassay for adult *A. holoxanthus*

Extraction of dislodgeable pesticidal residues: Leaf samples for quantification of dislodgeable residues were collected simultaneously with samples for parasite bioassay from each treatment plot in the field. Fifty 2.54 cm diam leaf discs were collected randomly from the 4 trees of each treatment replicate into prelabelled wide-mouthed glass jars by a leaf punch. Jars with leaf discs were placed on ice, transported to the laboratory, where they were held at 4°C, and were extracted within 10 h. The procedures for determination of dislodgeable residues of carbaryl and dicofol on foliage were modifications of methods described by Iwata et al. (1977). Briefly, 100 ml of water and 4 drops of a 1:50 dilution of 70% dioctylsulfosuccinate wetting agent were added to the collection jars. The capped jars were shaken on a reciprocating shaker at 200 RPM for 20 min. The liquid from the jar was poured into a 500 ml separatory funnel, while retaining the leaf discs in the jar and paying particular attention to transferring the associated leaf dust to the separatory funnel. This process was repeated. Fifty milliliters of methylene chloride then were added and the combined leaf disc extractions were added to a separatory funnel. The separatory funnel was shaken vigorously 10 times and the methylene chloride layer was removed into a round-bottomed flask containing 10g sodium sulfate. The methylene chloride extraction was repeated and the extracts combined. The methylene chloride was then evaporated to dryness with a rotary-vacuum-evaporator at 40°C. The residues were transferred to 10 ml volumetric flasks with three 1.5 ml acetone rinses of the evaporation flask and were brought to the mark with acetone. The extracts were transferred to 30 ml scintillation vials and stored at -17°C. Carbaryl and dicofol standards in acetone were stored with each extraction series to determine storage stability. Gas chromatography (GC) was performed on a Tracor 540 (Austin, TX 78725) gas chromatograph equipped with Ni^{63} electron capture detector. The column was a DB-5 fused silica megabore, film thickness 1.5μm with dimensions of 15m x 0.554mm (J&W Scientific, Folsom, CA 95630). Operating conditions were as follows: N_2; 30 ml/min column and 30ml/min detector makeup; injection temp. 235°C for carbaryl and 230°C for dicofol, column temp. 200°C and detector temp; 300°C for both carbaryl and dicofol. Each injection comprised 5μl. Analytical standards of Kelthane (TM) (98.0%, lot# 128-93c), 4.4' dichlorobenzophenone (99.0%, lot# 116-110B) and carbaryl (99.0%, lot# 143-9A) were from ChemService (Westchester, PA 19381-9941). Quantification was by a standard curve produced over the course of each analysis day by injecting a standard after every 5th sample.

Data analysis: Arcsine transformed survival data were analyzed with ANOVA and Fisher's least significant difference (LSD) test. To quantify relationships between mortality and pesticidal residues of dicofol and carbaryl, probit analyses and nonlinear regression 'Plateau model' were used respectively (SAS Institute, 1988).

Results

Mortality: Mortality of adult *A. holoxanthus* by carbaryl and dicofol residues after periods of field weathering is presented in figure 3.2. The parasites showed great sensitivity to carbaryl residues. During the 10 d post-treatment, carbaryl residues caused 100% parasite mortality. The mortality data represent 24 h of parasite exposure to treated leaves. However, during the first three days of bioassay, the parasites were dead after 4 to 5 h of exposure. By day 14 post-treatment, carbaryl residues did not kill all of the parasites within the 24 h exposure period. Surviving parasites after day 14 were very active. Mortality decreased quickly after 22 d and was equivalent to controls on the 27th day.

Dicofol residues affected *A. holoxanthus* differently than residues of carbaryl. The mortality of adult *A. holoxanthus* by dicofol 4 h after application was 33.6%, after 24 h it was 6.46%, which was not different from control mortality (6.25%). After 24 h, mortality of *A. holoxanthus* by dicofol showed no significant effect through day 31 when the experiment was terminated.

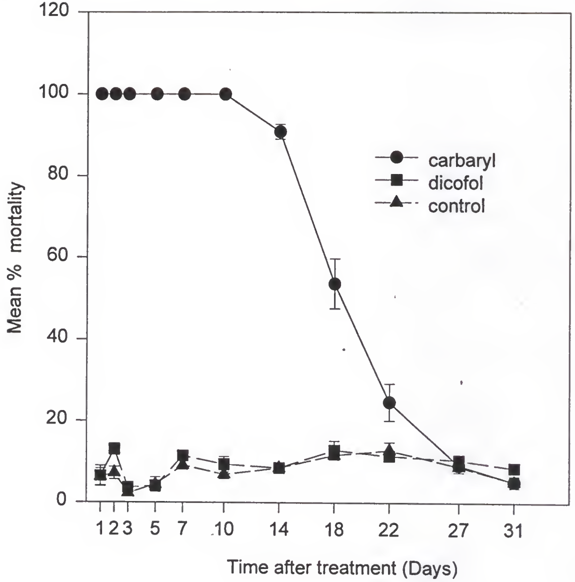

Figure 3.2. Mean % mortality of *Aphytis holoxanthus* cohorts exposed for 24 hours to treated leaves. Error bars represent standard error of mean.

Table 3.1: Mean percent mortality of female and male adult *Aphytis holoxanthus* 10 d after treatment application*.

Treatments	x̄ % ♀ dead ± SEM**	x̄ % ♂ dead ± SEM
carbaryl	36.63 ± 15.76	36.64 ± 18.37
dicofol	10.92 ± 0.87	12.34 ± 3.77
control	8.63 ± 1.38	10.41 ± 3.17

* 100% mortality of both sexes with carbaryl prior to day 10, see Fig. 2.
** Standard error of mean.

The population of adult *A. holoxanthus* in this experiment was female-biased, with 85.6% females (n=2240). Mean percent mortality of female and male adult *A. holoxanthus* after 10 d of carbaryl, dicofol and control treatments is given in Table 3.1.

The differences in overall mortality rates of female and male parasites did not depend upon treatment, time or their interactions, although males appeared to be slightly more sensitive to carbaryl than females.

Residue analyses: Extracuticular foliar residues of carbaryl and dicofol decreased as a function of time (Fig. 3.3). There were no pesticidal residues detected 2 d prior to the application of treatments. Gas chromatographic analyses indicated the peak retention time of carbaryl was 1.26 min and there was no hydrolysis to ∝-napthol in the field samples of carbaryl. The dicofol peak retention time was 1.67 min. Dicofol residues degraded more rapidly

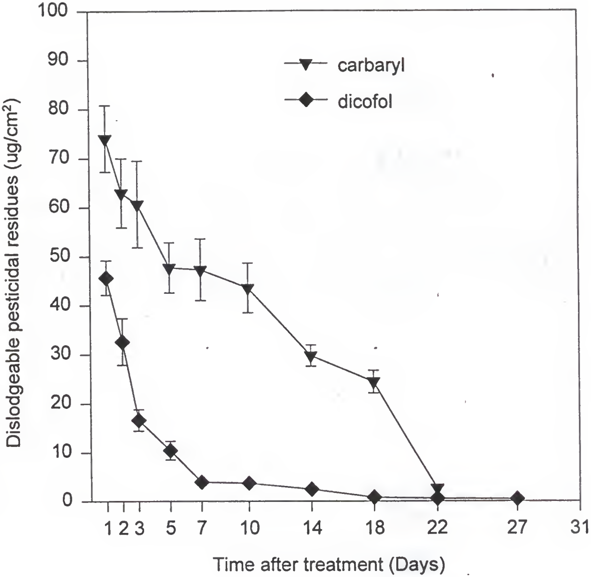

Figure 3.3. Relationship between quantity of carbaryl and dicofol dislodgeable residues and field weathering over time. Error bars represent standard error of the mean.

than did carbaryl residues on foliage. Both carbaryl and dicofol residues degraded very quickly on citrus leaves during the first 5 d, but from day 7 to 22, dicofol showed a more gradual pattern of degradation than carbaryl. Carbaryl residues were not detectable by day 27.

Interaction between dislodgeable residues and mean percent mortality of *Aphytis*: Laboratory bioassay results of adult *A. holoxanthus* on carbaryl-treated leaves were correlated with quantities of dislodgeable pesticidal residues over time as field-weathering of residues progressed. The mean mortality was 100% in carbaryl treatments within 10 d after application, during which residues ranged from 74.0 to 43.5 $\mu g/cm^2$ (Fig. 3.4). After 10 d, the mean percent mortality decreased very quickly to the control level, with carbaryl residues ranging from 29.4 to 2.65$\mu g/cm^2$. In contrast, mean percent mortality by dicofol residues after 24 h did not exceed 14% while residues decreased from 45.7 $\mu g/cm^2$ at 24 h to 0.46 $\mu g/cm^2$ recovered after 27 d (Fig. 3.5). Probit analysis of bioassay mortality and dicofol residue showed no correlation, indicating that dicofol had no impact on adult parasite survival.

The nonlinear regression of mortality versus carbaryl residues estimated with the plateau model showed that if the residues were $\leq t$, then the mortality was 1-b(t-residue), but if the residues were $>t$, then the mortality was 100 %,

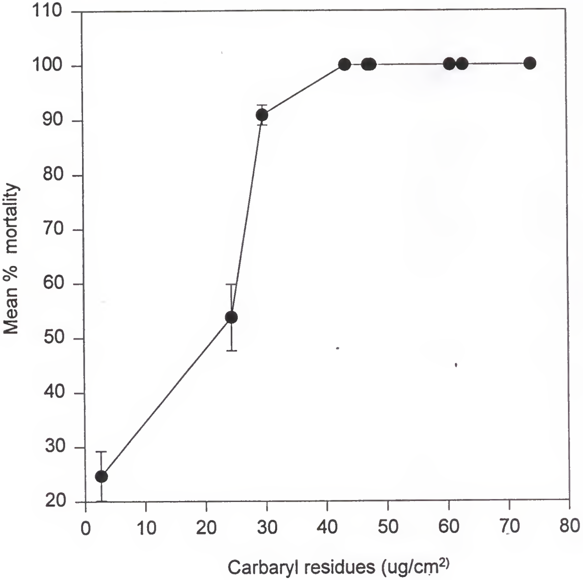

Figure 3.4. Mean % mortality of adult *Aphytis holoxanthus* vs carbaryl residues. Error bars represent standard error of the mean.

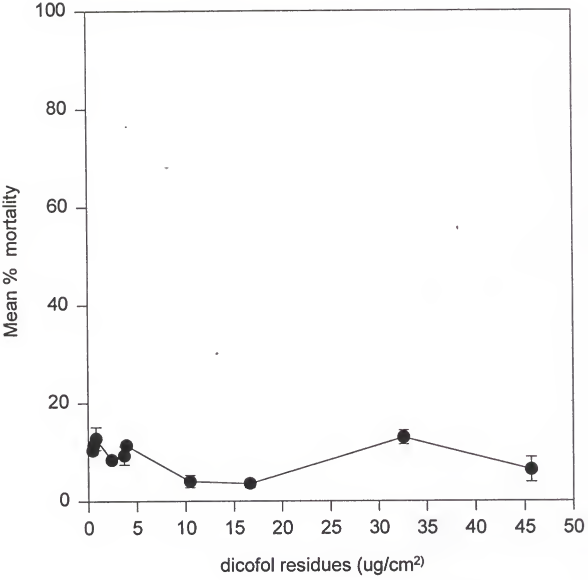

Figure 3.5. Mean % mortality of adult *Aphytis holoxanthus* vs dicofol residues. Error bars represent standard error of the mean.

where "b" is the slope of the line and "t" is the point where mortality reaches 100 %. In our experiments with carbaryl, b= 0.02, t=39.40 and r^2=0.98. This nonlinear regression model (plateau model) indicates that from 0 to 39.40μg/cm^2, the mortality increased at the rate of 2% mortality per unit(μg/cm^2), and at 39.40 μg/cm^2 the mortality is predicted to reach 100%. The LC_{50} and LC_{90} for carbaryl calculated by this model are 15.59μg/cm^2 and 34.61μg/cm^2, respectively.

Discussion

Bioassays of field-weathered residues, although faithfully representing the conditions of the particular treatments applied and tested, are not always a satisfactory picture of results with the same material and dosage under slightly different weathering conditions (Bartlett, 1953). Field residues of carbaryl in this study elicited 100% mortality of adult *A. holoxanthus* during the first 10 d. Debach and Bartlett (1951) demonstrated the virtual impossibility of maintaining effective populations of *Aphytis* in the continuous presence of synthetic organic insecticides, whether applied to control red scale or for other citrus pests. According to Bartlett (1953), new, persistent-residue citrus pest control materials destroyed natural enemies and caused increases of pests formerly held under biological control, and also have caused rapid flare-back of treated pests following initial control. Abdel-Rahman (1973) concluded that integration of malathion and biological control of red scale did not seem possible. Dean (1975) concluded that effective biological control of purple scale, *Lepidosaphes beckii* (Newman) by *A. lepidosaphes* in Texas was disrupted by carbaryl application. Carbaryl had the highest residual toxicity of all pesticides tested on a susceptible colony of *A. melinus* Debach in California (Spollen and Hoy 1993). In our study, mortality of *A. holoxanthus* by carbaryl residues declined to the control level on the 27th day.

Morse et al. (1987) reported that *A. melinus* mortality by field-weathered residues of dicofol was not significant. Similarly, our study showed that dicofol was toxic to *A. holoxanthus* only for the initial few hours after application.

The sex ratio of *A. holoxanthus* was not affected by carbaryl treatment. The mean mortality of males and females by carbaryl was not significantly different after 10 d. During the first 10 d of carbaryl treatment, there was 100% mortality of *Aphytis* from exposure to treated leaves. Male parasites showed greater sensitivity to carbaryl because males were found dead earlier in the 24 h exposure period than were female parasites. Additionally, only female parasites were able to survive after exposure to treated leaves on day 14 in the carbaryl treatment.

The analyses indicated that degradation of carbaryl and dicofol residues on citrus leaves was gradual. According to Nigg et al. (1979) 55% variation of the parathion residue behavior on citrus foliage was explained by time alone. In another study Thompson et al. (1979) concluded that there was rapid disappearance of Supracide (methidathion) dislodgeable residues during the 5-7 day period after spraying. The dislodgeable residues of Supracide comprised about 20% of the total residues, 80% being either absorbed or bound to the leaf surface so as not to be removed by a water detergent wash. Both carbaryl and dicofol residues, in our study, declined quickly during the initial 5 d period after application, but the degradation of carbaryl residues was more rapid after 10 d in comparison to dicofol residues. Dicofol residues were detectable even after 27 d, although they did not kill *Aphytis* after 24 h post-treatment.

Our data showed that field weathered carbaryl residues killed *A. holoxanthus* for a period of up to 22 d post-treatment under spring conditions in Florida. Dicofol residues, on the other hand, were less toxic to the adult parasites. These data suggest that carbaryl and dicofol might be intelligently used in pest management programs by considering the periodicity of their potential impacts on adult *Aphytis holoxanthus* and other important parasites of Florida citrus pests.

CHAPTER 4

EFFECTS OF PESTICIDAL DEPOSIT PATTERN ON RATE OF CONTACT AND MORTALITY OF *APHYTIS HOLOXANTHUS* DEBACH (HYMENOPTERA: APHELINIDAE)

Introduction

Biological control of Florida red scale (FRS) by the introduction of *Aphytis holoxanthus* has permanently reduced the pest status of FRS to the extent that citrus growers generally have forgotten that during the 1950s it was one of their most serious pests (Browning, 1994). This parasite searches actively on citrus foliage and fruit to find its armored scale host and can be negatively affected by pesticidal deposits due to high rates of contact associated with its walking on plant surfaces. The impact of pesticidal deposits on parasite survival may be dependent upon the pattern of deposition, i.e. size and number of deposits.

Different spray techniques are used to achieve spray deposition efficiency on citrus leaves to maximize control of target pests, but improvements in deposition may also severely disrupt host-parasite relationships. As a result of an increased ability to quickly and accurately measure the droplet size of released sprays, the effects of droplet size on deposit and on biological activity of the spray in relation to a specific crop pest can be more clearly defined (Akesson & Yates, 1989). The spray deposition efficiency of orchard air blast sprayers is generally considered to be low and is greatly influenced by droplet size (Reichard et al., 1977). New leaves have less surface waxes and showed slightly better deposition pattern than mature waxy leaves. Bottom surfaces of leaves, having a more rough texture, captured more droplets than the top surfaces (Salyani, 1988). The objective of this study was to characterize the effects of different pesticidal deposit patterns and sizes on rates of parasite contact during leaf searching, and resultant impacts on adult parasite mortality.

Materials and Methods

A laboratory study of pesticidal deposit pattern in relation to contact rate and mortality of *Aphytis holoxanthus* was conducted. Adult parasites were allowed to search on leaf surfaces containing various patterns of pesticidal deposits. The total amount of pesticide and carrier delivered was the same among all patterns. Parasite behavior was evaluated during searching, and rates of contact with pesticidal deposits were calculated for each deposit pattern.

Similarly, bioassays were conducted using each deposit pattern to determine the influence of pattern on parasite mortality induced by the pesticidal residues. These experiments were conducted under laboratory conditions of 24±2°C and about 60% R.H.

Behavior of *A. holoxanthus* on different deposit patterns: Four deposit patterns of carbaryl and untreated controls were replicated three times. The pesticidal treatment was carbaryl wettable powder (Sevin 80S: Rhone-Poulenc, Research Triangle Park, NC 27709) at 567g carbaryl per 378 liters of water (1.25 lb per 100 gal). Water served as the control. Kaolin clay (500 mg) was added to all solutions to improve the visibility of deposits. Deposit patterns of 1, 2, 5 and 10μl droplets were made with a 10-μl ±1% microdispenser (Drummond, USA). A total of 20μl of each solution was dispensed onto 2x1.7 cm 'Hamlin' orange rectangular leaf patches. The required number (20,1μl; 10,2μl; 4,5μl; or 2,10μl of droplets) in each replication was air dried for 90 minutes. The actual deposit size for each pattern was obtained by measuring the deposit through a microscope. Four deposits for each pattern were measured horizontally and vertically, and the product (LxW) was multiplied by a conversion factor of 73.9 to obtain the deposit area in micrometers (μm). Twenty adult *Aphytis holoxanthus* were exposed to each deposit patterns in each replication by confining them in a small ventilated petri dish for each single leaf patch. Each replication was recorded for 2 h by time lapse videography at 60 frames/sec (Panasonic, Japan). Observations were then interpreted and recorded during the first 10 min, the middle 10 min, and the final 10 min of the 2 h exposure period. In each case, the observations were made by analyzing the first 10 parasite searching events on each leaf patch. An event consisted of an individual adult parasite traversing the leaf patch. The total time on the patch and number of encounters with spray deposits were recorded during review of the video-taped exposures. Rates of contact were then calculated by dividing the number of contacts between the parasite and a deposit droplet, by the total time the parasite spent on the leaf patch. These data were analyzed by PROC GLM (SAS Institute, 1988), comparing the rates of contact for different deposit patterns during three time intervals (first 10 min, mid 10 min, and final 10 min).

Mortality of *A. holoxanthus*: The four deposit patterns described above (20,1μl; 10,2μl; 4,5μl; or 2,10μl droplets) for each treatment of carbaryl and control were repeated on 4.5cm leaf discs punched from field collected leaves and were air dried for 90 minutes. Four replicates of each treatment (pattern and presence/absence of carbaryl) were placed into ventilated plastic petri dishes of 4.9 cm diameter. The standard bioassay procedure as described in chapter 3 was then followed. After 24 h exposure to adults of *A. holoxanthus*, mortality data were collected from each deposit droplet pattern by detaching the petri dishes from the ventilation system and counting the live and dead parasites in each petri dish. The mean percent mortality was calculated and compared between deposit pattern and presence or absence of carbaryl. The data were analyzed with ANOVA and Fisher's least significant difference (LSD) test.

Results

Rate of contact by *A. holoxanthus*: As the number of droplets in the deposit pattern increased, the rate of parasite/deposit contact increased, even though total volume of deposit remained constant. The rate of contact was highest on leaf discs with 20 deposits in both the control and carbaryl treatments. Although the size of deposits increased as they become fewer, the rate of contact with larger deposits was lower than with smaller deposits that were more abundant. The droplet sizes and droplet volume for different deposit droplet numbers are given in table 4.1. According to PROC GLM (SAS Institute, 1988), the mean rate of contact to different numbers of deposits was highly significant ($F=13.16$; $df=11$; $P<0.0001$ and $r^2 = .92$), being directly proportional to the number of deposit droplets. Observations of different time intervals during the 2 h exposure indicated that the rate of contact within different intervals (first 10 min, mid 10 min, and final 10 min) for

Table 4.1. Mean rate of contact of *Aphytis holoxanthus* to different deposit numbers and sizes.

Trts.	Deposit #s.	drop vol. (μl)	$\bar{x}$ drop size (μm) (±SE)	$\bar{x}$ rate of contact (# of contact/sec) (±SE)		
				First 10 minute	mid 10 minute	final 10 minute
cont.	2	10	4247(95.3)	0.07(.04)	0.23(.08)	0.32(.06)
	4	5	3564 (145)	0.19(.06)	0.49(.08)	0.59(.05)
	10	2	2539(88.4)	0.41(.04)	1.01(.18)	0.68(.09)
	20	1	1939(38.4)	0.75(.13)	1.31(.39)	1.49(.28)
carb.	2	10	4893(61.5)	0.11(.03)	0.29(.16)	0.09(.03)
	4	5	3926(61.1)	0.16(.09)	0.39(.14)	0.15(.02)
	10	2	2631(48.6)	0.29(.07)	0.29(.19)	0.25(.09)
	20	1	2092(32.6)	1.07(.28)	0.69(.11)	0.67(.13)

SE= Standard error of mean

pesticide and non-pesticide treatments was also significant (F=6.77, df=2, P<.0107). Rates of contact with carbaryl droplets were higher during the first 10 min and decreased during the middle and final 10 min periods, in comparison to the control, where rates of contact increased during the middle and final 10 min, especially for patches with 20 deposited droplets, as shown in figure 4.1-4.3. Carbaryl treatments with 10 and 20 deposit droplets showed evidence of parasite effects during the middle 10 min and final 10 min periods. Adult parasites became sluggish and their mobility was decreased. Few sluggish or immobile parasites were observed on leaf patches with 2 or 4 deposits of carbaryl. Control treatments showed no evidence of negative effects on parasite activity during the whole 2 h exposure period. The parasites spent most of their time walking or grooming on the leaf surface, but responded to detection of deposits on the leaf surface by exhibiting antennation and probing behavior. Occasionally, parasites were observed to stop on deposit droplets, circle around them and tap or probe the margins of the deposits with their ovipositers. Contact with deposits appeared to be random. Parasites did not appear to be either attracted or repelled by the deposits in either the carbaryl or control treatments.

Mortality of *A. holoxanthus*: The bioassay indicated that mean percent mortality of *A. holoxanthus* in the carbaryl treatment was highest on leaf discs with smaller and more abundant deposits. The mean percent mortality in the carbaryl treatment on leaf discs with 20 and 10 deposits was 53.79% and 45.19% respectively. There was no mortality on leaf discs with 4 deposits of carbaryl. However, some parasites were sluggish and a few were immobile. Parasite mortality in deposit patterns without carbaryl (control) averaged 3.69% on 2 deposits and 1.19% on 10 deposits (Fig 4.4)indicating no significant effect of deposit size or number.

Discussion

According to Himel (1969), as the diameter of the average droplet size decreases, the total number of spray droplets increases markedly. This increases the potential for contact of the droplets with target insects. In another study Himel and Moore (1969) mentioned that most commercial spray devices produce spray droplets whose size (diameter) ranges from a few to hundereds of microns. The efficiency of any pesticide spray and the degree of control obtained is a function of the ultimate point of deposition of each of the pesticide spray droplets produced. In this study *A. holoxanthus* did not react differentially to the carbaryl and control deposit patterns. Random contact of *A. holoxanthus* with deposits in both the carbaryl and control treatment suggested that there was no obvious attraction or repellency by these deposits. The high rates of mortality of *A. holoxanthus* in the carbaryl treatments can be attributed to toxicity of carbaryl residues to these parasites. The high rates of contact of *A. holoxanthus* during the 1st interval of the 2 h exposure time resulted in slow walking and extended grooming and resting on the leaf patch. The low rates of contact in the pesticide treatments of 10 and 20 deposits at the middle 10 min and final 10 min periods of the 2 h exposure were due to the parasites' sluggish behavior. In contrast, the parasites on control deposits were very active throughout the 2 h exposure period.

Salyani and McCoy (1989) concluded that decreasing spray droplet size increased citrus rust mite control. Our study showed that, for a constant amount of pesticide applied, increased numbers of smaller droplet deposits enhanced coverage and resulted in increased contacts between actively searching *Aphytis holoxanthus* adults and static deposits. Greater mortality was experienced within treatments in which rates of contact were highest. Thus, improved coverage of foliar pesticidal applications may magnify the mortality rate of parasites.

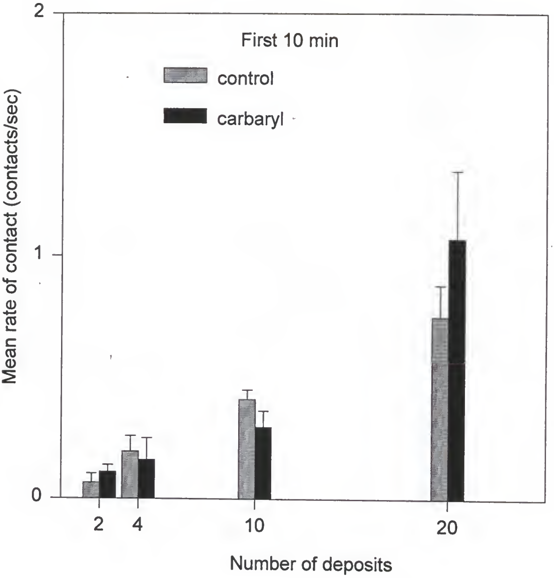

Figure 4.1. Mean rates of contact of adult *Aphytis holoxanthus* with deposits of different numbers & sizes during the first 10 min of the 2h exposure. Each pattern comprised 20 μl total solution delivered. Error bars represent standard error of the mean.

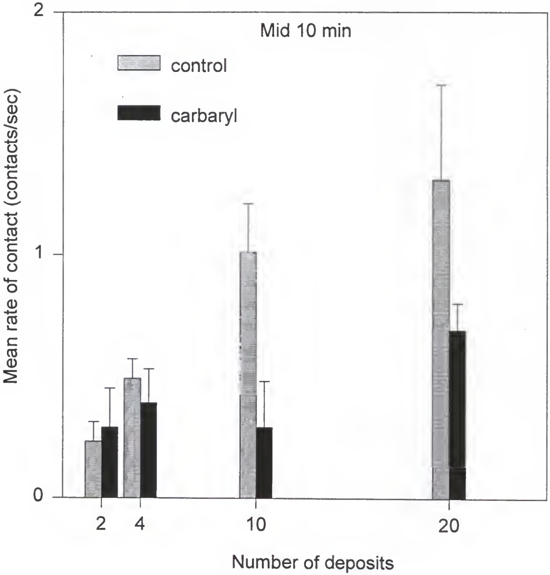

Figure 4.2. Mean rates of contact of adult *Aphytis holoxanthus* with deposits of different numbers & sizes during the mid 10 min of the 2h exposure. Each pattern comprised 20 μl total solution delivered. Error bars represent standard error of the mean.

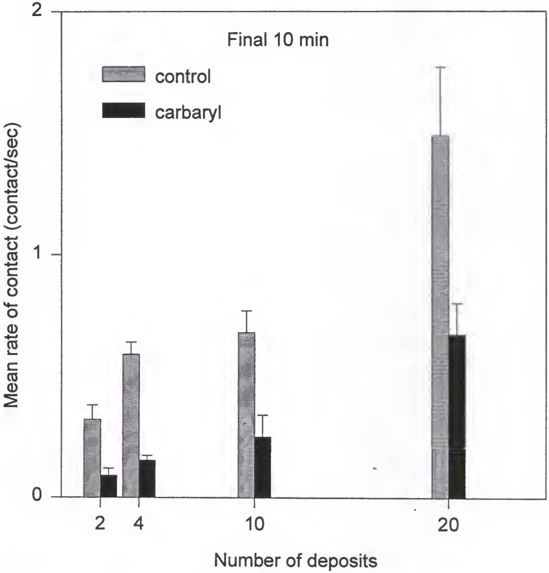

Figure 4.3. Mean rates of contact of adult *Aphytis holoxanthus* with deposits of different numbers & sizes during the final 10 min of the 2h exposure. Each pattern comprised 20 μl total solution delivered. Error bars represent standard error of the mean.

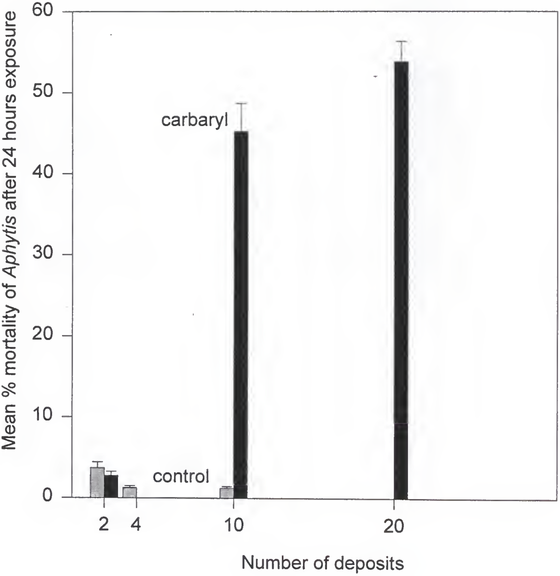

Figure 4.4. Mean % mortality of adult *Aphytis holoxanthus* after 24 hours exposure to treated leaves with different numbers & sizes of deposits. Error bars represent standard error of the mean.

CHAPTER 5
INDUCTION OF FLORIDA RED SCALE POPULATIONS THROUGH PESTICIDAL ELIMINATION OF *APHYTIS HOLOXANTHUS* DEBACH IN FLORIDA

Introduction

Armored scale insects (Homoptera: Diaspididae) constitute one of the most important groups of pests in agriculture (Rosen, 1973). Many species are especially destructive to fruit trees and ornamentals and their chemical control is often difficult and problematic. Being sedentary and often rather cryptic, numerous species have been inadvertently transferred with their host plants to new countries, where they have assumed the status of serious pests. Such invading species are targets for "classical" biological control (Rosen, 1973). Florida red scale (FRS) was a serious problem for grapefruit growers in Florida until the parasite, *Aphytis holoxanthus* was introduced in 1960. *Aphytis holoxanthus* was introduced into eight orange groves in central Florida, established quickly and in one year dispersed to most of the citrus-growing areas of the state (Clancy et al. 1963; Browning, 1994).

Species of *Aphytis* develop exclusively as primary ectoparasites of armored scale insects and are usually the most abundant and effective natural enemies of these pests. *Aphytis holoxanthus* is a highly effective natural enemy of the FRS, developing ectoparasitically on the soft body of its host, beneath the hard covering scale (Debach, 1960; Podoler et al., 1978).

Applications of broad-spectrum pesticides have been indicted in population increases of citrus pests formerly held under biological control (Bartlett, 1953). Therefore, the study reported here was designed to test the hypothesis that with knowledge of the periodicity of disruption by a broad-spectrum pesticide, a pest outbreak could be induced intentionally. The dynamics of such an upset could then be characterized.

Materials and Methods

The population dynamics of FRS and its parasitism by *A. holoxanthus* were determined through leaf and fruit sampling. Pesticides were applied at prescribed intervals to disrupt the host-parasite relationship, and comparisons were then drawn between treated and untreated plots.

Inoculation of FRS and *A. holoxanthus* in the field: Rearing techniques for FRS and *A. holoxanthus* have been described in chapter 3 and the same methodology was employed in this study. The FRS inoculation was begun in the first week of February, 1995. FRS crawlers were brushed from infested fruit of butternut squash, *Cucurbita maxima* Duchesne, onto a piece of 20x18 cm slippary paper and then transferred into a 20 ml glass vial. Less than 1 hr later, the FRS crawlers were released in a 'Hamlin' orange grove located at Davenport, Polk County, FL. Grove size was 2.3 hectares and the height of the 6 year old trees was 2 to 2.5 meters. Each tree within the grove was inoculated with FRS crawlers. Upper, middle and lower leaves were inoculated from East, West, North and South sections of the tree. Florida red scale was known to occur within this grove; the artificial infestation was made to foster uniform infestation of FRS. The crawlers were inoculated onto leaf surfaces using a fine camel-hair brush. Five to six leaves were inoculated at each location, each receiving 100-150 crawlers. Five to ten trees were inoculated daily depending upon the availability of FRS crawlers from the rearing operation, and through this procedure, all trees in the grove were inoculated within a two month period.

Aphytis holoxanthus inoculation followed establishment of FRS in the field. Wasps were aspirated from the parasite rearing system and 5-10 parasitic wasps were released near the scale insect colonies on each tree.

Pesticidal spray to disrupt host-parasite interaction: The field experiment utilized a randomized complete block design with four replications of three treatments. Treated plots consisted of 2 rows with 5 trees in each, totaling 10 treated trees per replicate. Each plot was buffered by at least 2 rows of trees, i.e. 4 trees on each side. The three treatment plots received: 1) carbaryl wettable powder (Sevin 80S: Rhone-Poulenc, Alexander Drive, Research Triangle Park, NC 27709) at 567g carbaryl per 378 liters of water (1.25 lb of carbaryl per 100 gal); 2) dicofol (Kelthane MF: Rohm & Haas, Philadelphia, PA. 19105) at 473 ml per 378 liters of water (one pint of dicofol per 100 gal); and 3) four randomly selected control plots received no pesticide. Pesticides were applied with a commercial air-blast sprayer.

Disruption of the host-parasite relationship was initiated by spraying carbaryl and dicofol on July 13, 1995. Five successive applications were made in 1995, with the three initial pesticidal applications timed at 14 d intervals as indicated by the bioassay results (Chapter 3). It was hypothesized from the bioassay study that a carbaryl application interval of 14 d would effectively eliminate *A. holoxanthus* in treated plots, while the same application interval for dicofol would have little or no effect on *A. holoxanthus*. Two additional applications were made on September 11 and October 23, 1995, and were scheduled at one month intervals to limit *A. holoxanthus* recolonization of carbaryl-treated plots. Both carbaryl and dicofol were applied to their respective plots on dates described above.

Leaf sampling procedure: Quantitative data on FRS population density fluctuations and corresponding changes in *A. holoxanthus* population density were collected by accumulating mortality and survival data of FRS and *A. holoxanthus* from the field site. Two leaf sampling techniques used in this experiment were modified from Debach et al. (1950).

Random leaf sampling: FRS population density was estimated monthly in each experimental plot. Three leaves were picked randomly from upper, middle and lower portions of each of the four trees sampled per plot. In the laboratory, scales were evaluated using a stereo microscope. The numbers and developmental stages of all live, dead, and parasitized FRS were recorded for each leaf after scale cover removal. Live scales were identified by their normal yellow color and the absence of any parasite egg, larva or pupa. All scales that were neither normal in appearance nor visibly parasitized were classified as "dead".

Selective leaf sampling: The methodology for collecting selective leaf samples and counting of FRS was the same as that described for random leaf sampling, except that in this case up to five leaves with FRS were collected from each of the 4 regularly sampled trees in each plot. Only those leaves infested with FRS were collected and evaluated. Whereas, random leaf sampling served to characterize host-plant/scale dynamics, selective leaf sampling was necessary to evaluate the interaction between FRS and *A. holoxanthus* in detail. Random and selective leaf samples were obtained routinely before and after pesticidal applications, with an interval of 14 days during initial months and 30-34 days from September through December.

The FRS densities and parasitism levels by *A. holoxanthus* were analyzed by using PROC GLM and likelihood ratio chi square test PROC FREQ (SAS Institute, 1988).

Fruit sampling: Florida red scale population densities on fruit were estimated monthly in each experimental plot. The scales on 20 randomly selected fruit from each of four regularly sampled trees in each plot were counted. Scale estimates were made on the whole fruit without removal from the tree. Florida red scale infestations on fruits were categorized as none(0), low (1-5 scales/fruit), medium (6-10), high (11-100) and severe ($>$ 100).

Total fruit drop and fruit infestation: In order to assess damage due to FRS, the total number of fruit on the trees, the number of fruit dropped and the number of infested fruit at the end of the harvest season were counted in each treatment. Data were collected on FRS infested and uninfested fruit on-tree and on the ground beneath each tree by using the categorical estimates described above.

Since general linear model techniques such as regression and ANOVA are not appropriate for categorized data, the 5 response levels were analyzed using a cumulative logit model (Harrison, 1996). In this approach, the ordinal response categories were ordered from "low" to "high" and then a logistic regression equation was used to predict the proportion of responses falling into these categories as a function of the terms in the model.

Results

Population dynamics of FRS: The results obtained in this experiment supported our hypothesis that pesticidal applications can disrupt a host parasite-relationship. High population densities of FRS were observed in the carbaryl treatment (Fig. 5.1). The population of FRS in the carbaryl treatment increased rapidly at the end of August as evidenced in the random leaf samples. This observation followed 3 spray applications at 14 day intervals. The FRS population then declined during September in the carbaryl treatment after no spray application between August 24 and September 5, but increased rapidly again on October 16 and November 13 following 2 spray applications during September and October.

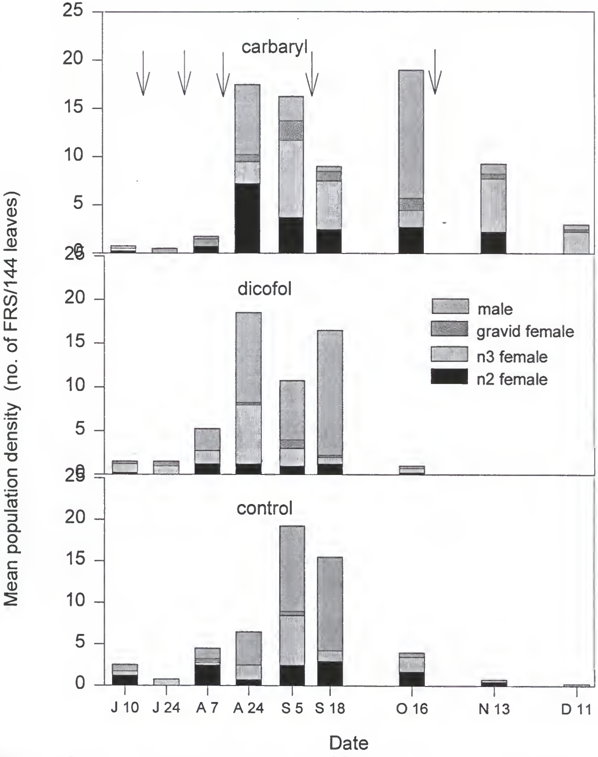

Figure 5.1. Effects of carbaryl and dicofol on population dynamics of different stages of Florida red scale in random leaf samples of `Hamlin' orange. Arrows indicate treatment application dates.

The general increase in population density of FRS in all of the treatments during summer was not uniform as measured in random leaf samples (Fig. 5.1). The population of FRS peaked during September and declined generally afterward in the untreated control, whereas, in the dicofol treatment, the population density of FRS was high during August and September and declined rapidly thereafter.

The % leaf infestation with FRS was generally low in all of the treatments, varying from 0 to 26% (Fig. 5.2). Leaf infestation increased in all of the treatments after July 24. The carbaryl treatment had a higher leaf infestation than the dicofol and control treatments. The highest leaf infestation (26%) was found in the carbaryl treatment on August 21 followed by the dicofol (13%) and the control(4%) treatments. A quick drop in leaf infestation, i.e., below 10%, in the carbaryl and the dicofol treatments was observed on September 5, which coincided with absence of spray applications during the period August 11 to September 10. Leaf infestation increased again in the carbaryl treatment (18%) on October 16 in comparison to the dicofol (3%) and control (4%) treatments. Leaf infestation by FRS declined rapidly in all of the treatments during the last two months of the year. The lowest leaf infestation was found in the control treatment, which remained below 6% throughout the experiment.

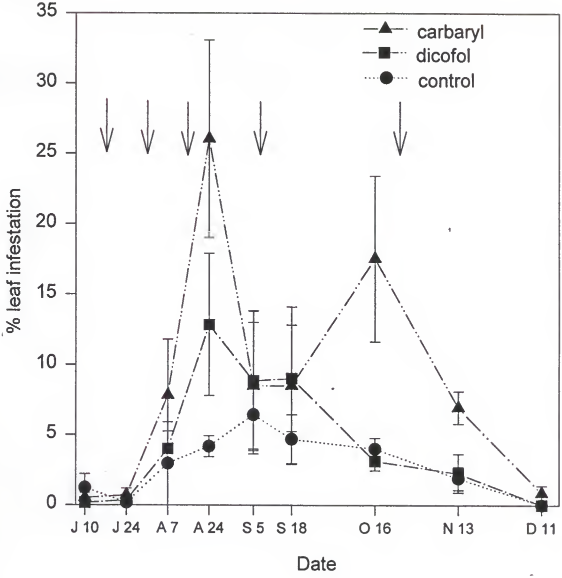

Figure 5.2. Effects of carbaryl and dicofol on percent 'Hamlin' orange leaf infestation with Florida red scale. Error bars represent standard error of the mean and arrows indicate treatment application dates.

Florida red scale was observed to be concentrated on small patches on each tree. Male scale appeared to be more abundant on upper surfaces of the leaves than the female stages, which were observed mostly on lower surfaces.

Mean densities of live female scales (2nd instar nymph (n2), pre-reproductive stage (n3) and gravid) were higher in the carbaryl treated plots than in the dicofol and control treatments (Fig. 5.1). The 2nd instar female densities were significantly different between the treatments ($F= 3.60$; $df= 2$; $P < 0.03$), being highest in the carbaryl treatment on August 24. This was followed by a rapid decline on September 5 and September 18 (Table 5.1 and Fig. 5.1). Female n2 density remained unchanged from August 7 through September 18 in the dicofol treatment. The female n2 densities in the control treatment were erratic from July to November. The overall mean densities of female n2 were 0.93 ± 1.10 in the carbaryl; 0.43 ± 0.31 in the dicofol; and 0.66 ± 1.03 in the control treatments.

Reproductive stages (n3+gravid females) are likely to give the best estimate of population change and are probably the predictor of the level of leaf and fruit infestations of citrus by subsequent FRS generations (Rosen and Debach, 1979). The mean density of FRS reproductive stages increased during July to September in all of the treatments and generally declined through later months of the year (Fig. 5.3). Decline in densities of n3+gravid females was

Table 5.1. Mean density of live n2 Florida red scale per 144 leaves on different dates in random leaf samples of `Hamlin' orange, n=12.

Date	control Mean^{x} ± SEM^{y}	dicofol Mean^{x} ± SEM^{y}	carbaryl Mean^{x} ± SEM^{y}
July 10*	1.25^{a} ± 0.94	0.25^{a} ± 0.25	0.25^{b} ± 0.25
July 24*	0	0	0
August 7*	2.5^{a} ± 2.5	1.25^{a} ± 0.75	0.75^{b} ± 0.4
August 24	0.75^{a} ± 0.47	1.25^{a} ± 0.94	7.25^{a} ± 2.71
September 5*	2.5^{a} ± 1.55	1.0^{a} ± 0.4	3.75^{ab} ± 2.25
September 18	3.0^{a} ± 2.38	1.25^{a} ± 0.4	2.5^{b} ± 2.17
October 16*	1.75^{a} ± 1.18	0.25^{a} ± 0.25	2.75^{b} ± 1.03
November 13	0.5^{a} ± 0.28	0	2.25^{b} ± 1.03
December 11	0	0	0

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988).

[y] Standard error of mean.

* Dates followed by pesticidal application.

erratic in the carbaryl treatment, with a high density observed on November 13 in random samples. The densities of n3 and gravid females were also significantly different between sample dates (F=7.35; df= 8; $P < 0.0001$)(Table 5.2 & 5.3). The mean scale densities per 144 leaves of n3 (8.0 ± 1.41) and gravid females (2.0 ± 1.35) were highest on September 5. The carbaryl treatment had the highest mean density of combined n3+gravid females (1.74 ± 0.49), greater than dicofol (0.87 ± 0.56) and the untreated (0.71 ± 0.83) plots (Table 5.4).

Table 5.2. Mean density of live n3 Florida red scale on different dates in random leaf samples of `Hamlin' orange, n=12.

Date	control Mean^X ± SEM^Y	dicofol Mean^X ± SEM^Y	carbaryl Mean^X ± SEM^Y
July 10*	0.5^b ± 0.5	1.0^b ± 0.70	0.25^c ± 0.25
July 24*	0.75^b ± 0.75	1.0^b ± 1.0	0
August 7*	0.25^b ± 0.25	1.5^b ± 1.19	0
August 24	1.75^b ± 1.75	6.75^a ± 4.02	2.25^{bc} ± 1.10
September 5*	6.00^a ± 1.95	2.00^b ± 0.95	8.0^a ± 1.41
September 18	1.25^b ± 0.75	0.75^b ± 0.47	5.0^{ab} ± 2.97
October 16*	1.75^b ± 1.75	0.5^b ± 0.28	1.75^{bc} ± 0.47
November 13	0	0	5.5^{ab} ± 3.1
December 11	0.25^b ± 0.25	0	2.25^{bc} ± 1.10

^{X} Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P< 0.05$ (SAS Institute,1988)

^{Y} Standard error of mean.

* Dates followed by pesticidal application.

Male FRS densities were generally higher in all of the treatments than female stages. Male FRS densities increased after July 24 and reached their highest level in the control (11.25 ± 4.26) and dicofol (14.25 ± 7.86) treatments on September 18 and in the carbaryl treatment (13.25 ± 10.96) on October 16 (Table 5.5). While treatments had no significant effect on male scale densities, these densities varied significantly between sample dates ($F=6.33$; $df=8$; $P< 0.0001$). There were no male FRS recovered from July 24 random leaf samples.

Table 5.3. Mean density of live gravid female Florida red scale on different dates in random leaf samples of `Hamlin' orange, n=12.

Date	control Mean[x] ± SEM[y]	dicofol Mean[x] ± SEM[y]	carbaryl Mean[x] ± SEM[y]
July 10*	0	0	0
July 24*	0	0.50^{ab} ± 0.05	0.5^{ab} ± 0.28
August 7*	0.50^{a} ± 0.50	0	0.75^{ab} ± 0.47
August 24	0	0.25^{b} ± 0.25	0.75^{ab} ± 0.75
September 5*	0.50^{a} ± 0.50	1.0^{a} ± 0.40	2.0^{a} ± 1.35
September 18	0	0.25^{b} ± 0.25	1.0^{b} ± 0.40
October 16*	0	0	1.25^{ab} ± 0.94
November 13	0	0	0.5^{ab} ± 0.28
December 11	0	0	0.25^{ab} ± 0.25

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

The intensive data from the selective leaf samples supported the random leaf sample findings. Higher leaf infestations of FRS in the carbaryl treated plots were evident during summer in comparison to dicofol and control. Moreover, Florida red scale infested leaves were more visible in the carbaryl plots than in the dicofol and control plots. The relative proportion of different life stages in the selective leaf samples varied between the treatments (Fig. 5.4). There was no consistent pattern of increase or decrease in different life stages of FRS in any

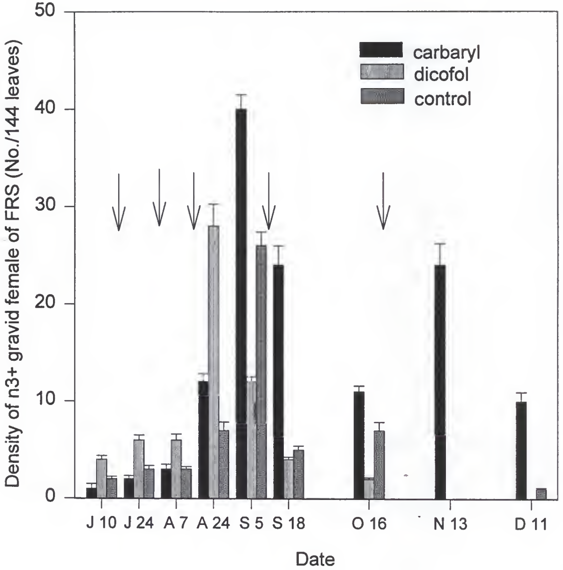

Figure 5.3. Effects of carbaryl and dicofol on 3rd instar and gravid female densities of Florida red scale in random leaf samples of `Hamlin' orange. Error bars represent standard error of the mean and arrows indicate treatment application dates.

Table 5.4. Mean densities of live 2nd instar nymph, 3rd instar female, gravid female and n3+gravid females of Florida red scale per 144 leaves in random leaf samples of `Hamlin' orange, n=36.

Female stages	Treatments	Mean[x] ± SEM[y]
n2	carbaryl	$0.93^{a} \pm 1.10^{*}$
	dicofol	$0.43^{b} \pm 0.31$
	control	$0.66^{ab} \pm 1.03$
n3	carbaryl	$1.19^{a} \pm 1.13^{*}$
	dicofol	$0.68^{b} \pm 0.95$
	control	$0.64^{b} \pm 0.88$
gravid	carbaryl	$0.56^{a} \pm 0.53^{**}$
	dicofol	$0.19^{b} \pm 0.16$
	control	$0.08^{b} \pm 0.11$
n3 + gravid	carbaryl	$1.74^{a} \pm 0.49^{**}$
	dicofol	$0.87^{b} \pm 0.56$
	control	$0.71^{b} \pm 0.83$

[y] SE= Standard error of mean.

[x] Means with different letters within each stage are significantly different using Fisher's LSD at * $P < 0.05$ and ** $P < 0.0001$.

of the treatments. Neither treatments nor time of sampling affected the relative proportions of different life stages of FRS.

Parasitism of FRS by *A. holoxanthus*: Parasitism of FRS tended to increase with the increase in FRS population. The effect of sample dates on parasitism of n3 female and gravid female FRS was evident from the selective samples (Table 5.6 & 5.7). There was a significant effect of sample

Table 5.5. Mean density of live male Florida red scale per 144 leaves on different dates in random leaf samples of `Hamlin' orange, n=12.

Date	control		dicofol		carbaryl	
	Mean^{x}	± SEM^{y}	Mean^{x}	±SEM^{y}	Mean^{x}	± SEM^{y}
July 10*	0.75^{b}	±.47	0.25^{b}	±0.25	0.25^{b}	±0.25
July 24*	0		0		0	
August 7*	1.25^{b}	±1.25	2.5^{b}	±1.65	0.25^{b}	±0.25
August 24	4.00^{ab}	±1.78	10.25^{ab}	±6.14	7.25^{ab}	±5.70
September 5*	10.25^{a}	±7.08	6.75^{ab}	±5.75	2.50^{ab}	±1.32
September 18	11.25^{a}	±4.26	14.25^{a}	±7.86	0.05^{b}	±0.05
October 16*	0.5^{b}	±0.28	0.25^{b}	±0.25	13.25^{a}	±10.96
November 13	0.25^{b}	±0.25	0		1.00^{b}	±0.70
December 11	0		0		0.5^{b}	±0.5

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

date ($F= 7.22$; $df= 7$; $P < 0.0001$) on n3 and ($F= 3.23$; $df= 7$; $P < 0.007$) on gravid female FRS parasitism in selective leaf samples. The parasitism of n3 female FRS was higher in the control (1.16 ± 0.44) and dicofol (0.75 ± 0.34) treatments than the carbaryl treatment (0.33 ± 0.13)on September 18 (Table 5.6). However, parasitism of n3 females was higher on October 16 in the carbaryl treatment (2.41 ± 1.31) than the dicofol (0.33 ± 0.19) or control (0.33 ± 0.23) treatments, possibly indicative of a rebound in parasitism as *Aphytis* spp recolonized carbaryl plots.

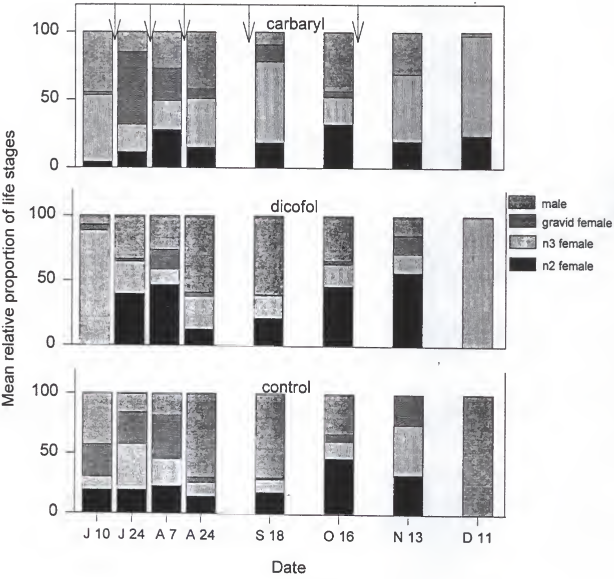

Figure 5.4. Effects of carbaryl and dicofol on mean relative proportion of different life stages of Florida red scale in Selective leaf samples of `Hamlin' orange. Arrows indicate treatment application dates.

Table 5.6. Mean parasitism of n3 Florida red scale on different dates as measured in selective leaf samples of `Hamlin' orange, n=4.

Date	control Mean^x ± SEM^y	dicofol Mean^x ± SEM^y	carbaryl Mean^x ± SEM^y
July 10*	0.16^b ± 0.09	0.08^b ± 0.08	0
July 24*	0.08^b ± 0.08	0	0
August 7*	0	0	0
August 24*	0.25^b ± 0.25	0.08^b ± 0.08	0
September 18	1.16^a ± 0.44	0.75^a ± 0.34	0.33^b ± 0.13
October 16*	0.33^b ± 0.23	0.33^{ab} ± 0.19	2.41^a ± 1.31
November 13	0.33^b ± 0.23	0.16^b ± 0.16	0
December 11	0	0.16^b ± 0.16	0.58^b ± 0.28

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

The highest parasitism of gravid females in the selective samples was observed on August 24 in all of the treatments (Table 5.7). The control had higher parasitism of gravid females (0.33 ± 0.23) than in the dicofol (0.25 ± 0.15) and the carbaryl (0.16 ± 0.16) treatments on August 24. There was no parasitism of gravid females in selective leaf samples on July 24, August 7, November 13 and December 11 in any of the treatments.

Parasitism of female n3 FRS in random leaf samples was first observed on August 24 in the carbaryl treatment and on September 5 in the control and dicofol treatments (Table

Table 5.7. Mean parasitism of gravid Florida red scale on different dates as measured in selective leaf samples of 'Hamlin' orange, n=4.

Date	control Mean[x] ± SEM[y]	dicofol Mean[x] ± SEM[y]	carbaryl Mean[x] ± SEM[y]
July 10*	0.16^{ab} ± 0.16	0.16^{a} ± 0.16	0
July 24*	0	0	0
August 7*	0	0	0
August 24*	0.33^{a} ± 0.23	0.25^{a} ± 0.15	0.16^{a} ± 0.16
September 18	0	0.08^{a} ± 0.08	0
October 16*	0	0.08^{a} ± 0.08	0.16^{a} ± 0.16
November 13	0	0	0
December 11	0	0	0

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

5.8). There was a significant effect of sample dates on parasitism of this stage (F= 6.54; df= 8; $P < 0.0001$). The mean rate of female n3 parasitism was highest on October 16 in the carbaryl treatment (1.25 ± 0.39). There was no parasitism of n3 female FRS reported in random leaf samples on July 24 and August 7 in any of the treatments. Declines in parasitism were not gradual but rather erratic.

Mean parasitism of gravid female FRS was significantly different on different leaf sampling dates (F= 9.12; df= 8; $P < 0.0001$). Parasitism of gravid female FRS was first observed in random leaf samples on September 5 in the

Table 5.8. Mean parasitism of n3 Florida red scale on different dates as measured in random leaf samples of `Hamlin' orange, n=12.

Date	control Mean[x] ± SEM[y]	dicofol Mean[x] ± SEM[y]	carbaryl Mean[x] ± SEM[y]
July 10*	0	0.08^{c} ± 0.08	0.08^{b} ± 0.08
July 24*	0	0	0
August 7*	0	0	0
August 24	0	0	0.08^{b} ± 0.08
September 5*	0.25^{ab} ± 0.15	0.58^{a} ± 0.08	0.25^{b} ± 0.15
September 18	1.08^{a} ± 0.8	0.41^{ab} ± 0.2	0.25^{b} ± 0.15
October 16*	0.25^{ab} ± 0.25	0.16^{bc} ± 0.16	1.25^{a} ± 0.39
November 13	0.08^{b} ± 0.08	0.08^{c} ± 0.08	0.33^{b} ± 0.13
December 11	0	0	0.08^{b} ± 0.08

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P< 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

control, on September 18 in the dicofol, and October 16 in the carbaryl treatments (Table 5.9). There was no parasitism of gravid female FRS observed in random samples prior to September 5 or on December 11 in any of the treatments.

Parasitism of reproductive stages of FRS combined (n3+gravid females) varied through out the year (Fig. 5.5). High n3+gravid female FRS densities during August and September in the control and dicofol treatments resulted in the highest rate of parasitism on September in these

Table 5.9. Mean parasitism of gravid female Florida red scale on different dates as measured in random leaf samples of `Hamlin' orange, n=12.

Date	control Mean^{x} ± SEM^{y}	dicofol Mean^{x} ± SEM^{y}	carbaryl Mean^{x} ± SEM^{y}
July 10*	0	0	0
July 24*	0	0	0
August 7*	0	0	0
August 24	0	0	0
September 5*	0.33^{a} ± 0.13	0	0
September 18	0	0.08^{a} ± 0.08	0
October 16*	0	0	0.08^{a} ± 0.08
November 13	0.08^{b} ± 0.08	0	0
December 11	0	0	0

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P< 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

treatments. Mean percent parasitism of n3+ gravid females in the random leaf samples remained lower in the carbaryl treatment in September than the dicofol and control treatments, possibly resulting from the 3 spray applications made prior to this date (Fig. 5.5). Parasitism of n3+gravid female FRS in the carbaryl treatment was higher (2.57 ± 1.47) on October 16 than the dicofol (0.41 ± 0.27) and control (0.33 ± 0.23) treatments following no spray applications during September 12 to October 23 period. This may reflect a response by parasites to high densities of

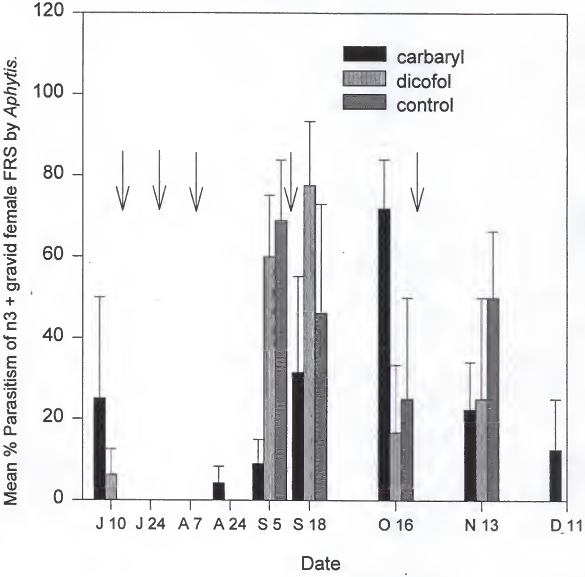

Figure 5.5. Effects of carbaryl and dicofol on parasitism of 3rd instar and gravid females of Florida red scale in random leaf samples of `Hamlin' orange. Error bars represent standard error of the mean and arrows indicate application dates.

Table 5.10. Mean parasitism of male Florida red scale on different dates as measured in random leaf samples of `Hamlin' orange, n=12.

Date	control Mean[x] ± SEM[y]	dicofol Mean[x] ± SEM[y]	carbaryl Mean[x] ± SEM[y]
July 10*	0.08[b] ± 0.08	0	0
July 24*	0	0	0
August 7*	0.08[b] ± 0.08	0	0
August 24	0.41[b] ± 0.31	0.33[ab] ± 0.23	0
September 5*	1.58[a] ± 0.55	0.58[a] ± 0.58	0.58[a] ± 0.25
September 18	0.25[ab] ± 0.25	0	0
October 16*	0.08[b] ± 0.08	0.08[b] ± 0.08	0
November 13	0	0	0
December 11	0	0	0

[x] Means followed by different letters (within column) are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988)

[y] Standard error of mean.

* Dates followed by pesticidal application.

Table 5.11. Mean overall parasitism of male Floriad red scale by *A.holoxanthus* in different treatments as measured in random leaf samples of `Hamlin' orange, n= 36.

Treatments	Mean[x] ± SEM[y]
carbaryl	0.06[b] ± 0.03
dicofol	0.11[b] ± 0.10
control	0.28[a] ± 0.15

[y] SE= Standard error of mean.

[x] Means followed by different letters are significantly different using Fisher's LSD test at $P < 0.05$ (SAS Institute,1988).

n3+gravid female FRS in September. There was no parasitism recorded in any of the treatments on July 24 and August 7. There was little or no parasitism observed on n2 female FRS.

Mean parasitism of male FRS in random leaf samples was higher (1.58 ± 0.55) in the control on September 5 than the dicofol (0.58 ± 0.58) and the carbaryl (0.58 ± 0.25) treatments (Table 5.10). Parasitism of male FRS in the carbaryl treatment was recorded only on September 5. Mean parasitism of male FRS was significantly different between the treatments ($F= 3.72$; $df= 2$; $P < 0.03$) (Table 5.11), with parasitism highest in the control (0.28 ± 0.15) followed by dicofol (0.11 ± 0.10) and carbaryl (0.06 ± 0.03) treatments.

On-tree fruit infestation: Fruit sampling for FRS infestation was begun in September, 1995, and overall, the majority of fruit in all the treatments was uninfested (Table 5.12). The carbaryl plots consistently had the highest infestations of FRS, followed by dicofol and the untreated control. The highest percentage of fruit infestation from FRS in the carbaryl treatment was observed on October 30, with substantial infestation in low (10.94%) and high (33.75%) categories, and on September 30 in the medium (9.82%) infestation category. On any particular day, and any split of "low damage" vs "high damage" categories, the likelihood ratio chi square (G^2) values were: control (2.90)> dicofol (2.40)> carbaryl (1.08), indicating that the untreated control had the lowest proportion of fruit in a

Table 5.12. Percent on-tree fruit infestation by Florida red scale in random fruit samples of `Hamlin' orange during the period from September to December,1995.

Sample date	Trts.	None[a] (%)	Low[b] (%)	Medium[c] (%)	High[d] (%)	Severe[e] (%)
Sept. 5	control	98.12	1.56	0.0	0.3	0.0
	dicofol	94.06	2.19	0.93	2.82	0.0
	carbaryl	89.38	4.06	2.19	4.38	0.0
Sept.30	control	94.06	2.5	1.25	2.19	0.0
	dicofol	89.69	4.38	1.88	4.06	0.0
	carbaryl	64.29	4.76	9.82	21.13	0.0
Oct. 30	control	85.63	5.94	4.38	3.44	0.63
	dicofol	88.13	5.01	2.19	4.38	0.31
	carbaryl	53.75	10.94	1.25	33.75	0.31
Nov. 27	control	91.27	2.19	2.82	3.75	0.0
	dicofol	84.40	4.38	5.94	5.31	0.0
	carbaryl	64.39	5.64	8.13	20.94	0.94
Dec. 27	control	93.13	0.63	3.13	2.5	0.63
	dicofol	90.94	0.3	3.45	4.68	0.63
	carbaryl	73.75	0.94	7.50	15.31	2.50
Seasonal mean%	control	92.44	2.56	2.31	2.44	0.25
	dicofol	89.44	3.25	2.88	4.25	0.19
	carbaryl	69.06	5.26	5.82	19.12	0.74

[a] (0), [b] (1-5), [c] (6-10), [d] (11-100), [e] (> 100).

Table 5.13. Mean % `Hamlin' orange fruit dropped with and without Florida red scale infestation prior to the harvest season.

	% of fruit on-tree		% of fruit on-ground	
control	91.14		8.86	
dicofol	87.4		12.6	
carbaryl	89.3		10.7	
	% FRS infestation		% FRS infestation	
	uninfested	infested	uninfested	infested
control	93.13	6.87	95.59	4.74
dicofol	90.94	9.06	90.95	8.81
carbaryl	73.75	26.25	83.74	16.26

"high" damage category, followed by dicofol and carbaryl. With "K" (5) ordinal categories, there were (K-1) possible divisions of low and high categories. The logistic regression model estimates were averaged over all (K-1) possible splits to provide final estimates for differences in fruit infestation severity.

Effects of scale infestation on fruit drop: A count of the total number of fruit on the tree versus total number of fruit dropped (fruit with and without FRS) was made on December 14, 1995. The amount of fruit drop was relatively low in all the treatments (Table 5.13). However, the likelihood ratio chi square test PROC FREQ (SAS Institute, 1988) showed that carbaryl (10.7%) and dicofol (12.6%) had significantly higher fruit drop than the control (8.86%)(G^2= 8.30, df= 2, $P < 0.004$). The proportion of fruit on the ground which was infested with FRS was also significantly higher in the carbaryl and the dicofol treatments than the control (G^2=21.09, df= 2, $P < 0.001$). The on-ground fruit infestation rates were not significantly different between the carbaryl and dicofol treatments.

Discussion

The first inoculation of FRS was made in February, 1994 and parasites were first released in April, but populations of FRS were low throughout 1994. The FRS was again inoculated successfully in February-March, 1995 and parasites were released in April, 1995. Florida red scale and *A. holoxanthus* were observed in all the treatments at the time of first leaf sampling on July 10, 1995. Populations of FRS were observed in small patches on the trees. Occasionally, there was little or no FRS on one side of the tree while the opposite side was heavily infested. Florida red scale populations were most often encountered on dark green, mature leaves. We observed little or no FRS infestation on old or dry leaves. Different stages of FRS (male and n2, n3 and gravid female) were found on individual leaf samples with high male densities. Florida red scales were found on both sides of the leaf with male FRS being more scattered on the leaf surfaces than female FRS, which were feeding close to other female stages of FRS. In some cases FRS were clustered beneath 2 or 3 scale covers of previous generations. There were many occasions when no live scales were found beneath the scale covering, and these scales were either dry or had been preyed upon.

We successfully disrupted the host-parasite relationship in the treatment using a pesticide toxic to *A. holoxanthus*. Our results showed that carbaryl applications increased Florida red scale populations. The elimination of *A. holoxanthus* in the carbaryl-treated plots during early months of the experiment resulted in increased FRS populations. There was a general increase in FRS population in all the treatments during summer. The heavy infestation of FRS was most evident on carbaryl-treated leaves.

According to Dean (1982), pesticides have been associated with the disruption of *A. holoxanthus* in Texas citrus, with the result that FRS occasionally became local economic pest. Dean (1982) reported that the most pronounced increases in scale populations occurred during July to September, although early increase could occur as early as May. Parasite populations varied considerably, but usually increased during the latter four months of the year. The most apparent increases in FRS populations in Texas were related to decreases in *Aphytis* following the use of pesticides (Dean, 1982). Our study documented increases in scale populations during July to September, but we did not observe any increase in FRS populations during May.

Ben-Dov and Rosen (1969) concluded that increased parasitism of California red scale by *Aphytis* in Israel in pesticide-treated plots during July, August and September might be ascribed to the attraction of *Aphytis* females to the increased host scale populations in the treated plots, the rapid developmental rate of the parasites, the presence of a large reservoir of natural enemies on untreated trees in the immediate vicinity and the quick decomposition of pesticides during these months. Our results showed that parasites recovered quickly in carbaryl-treated plots even after four pesticide applications within a two month period, provided there were high numbers of FRS in these plots. It is not clear whether *Aphytis holoxanthus* was recolonized from adjacent untreated trees or was recovering from populations which survived the four treatments of carbaryl.

Dean (1977) concluded that dicofol produced no measurable decrease in parasites or increase in pest species following spray application, which our results support. In the present study, the FRS population was very low on leaves at the end of December, 1995 and was almost undetectable in January after heavy defoliation followed a freeze in December, 1995. This precluded continuation of the study which had been designed to characterize recovery from pesticide-induced scale upset.

Fruit infestation was first observed during September 1995. Percent uninfested fruit were consistently high in all the treatments (Table 5.12). There was a general increase in fruit infestation through latter months of the year as fruit expanded in size and matured. Fruit infestation was higher in the carbaryl treatment than in the dicofol and control treatments. Some fruit were heavily infested compared to other fruit on the same tree which had low or no infestation of FRS. The heavy infestation of FRS also caused greater fruit drop in the carbaryl treatment.

In general, our results showed that repeated applications of a pesticide with high toxicity to parasites increased population densities of FRS most likely by eliminating *A. holoxanthus*. The recovery of *A. holoxanthus* in treated plots with high FRS densities was rapid when the pesticidal pressure was lifted. Our results suggest that integration of pesticides into pest management programs is possible, if the dynamics of pesticide application frequency, host population response and parasite recolonization are understood.

CHAPTER 6
CONCLUSIONS

The results obtained in this study reiterate the importance of considering the impact of pesticidal application practices on biological control agents in integrated pest management programs. It has been demonstrated earlier that *Aphytis holoxanthus* has the potential to keep FRS populations at minimal levels. However, our results indicated that disruption induced by pesticides toxic to *Aphytis holoxanthus* severely limited the effectiveness of this parasite.

Field-weathered residues of carbaryl in our laboratory study elicited 100% mortality of adult *A. holoxanthus* during the first 10 days following treatment (Chapter 3). According to Bartlett (1953), persistent residue from citrus pest control materials, by destroying natural enemies, can cause increases of pests formerly held under biological control, and are responsible for rapid flare-back of treated pests following initial control. In our study, field-weathered carbaryl residues killed *A. holoxanthus* for a period of up to 22 days post treatment under spring conditions in Florida. The degradation of both carbaryl and dicofol residues on citrus leaves was similar under field conditions, with carbaryl and dicofol residues declining quickly during the initial 5 day period after application. The degradation of carbaryl residues was more rapid after 10 days in comparison to dicofol residues. Morse et al. (1987) reported that *A. melinus* mortality by field weathered residues of dicofol was not significant. Similarly, our study showed that dicofol was toxic to *A. holoxanthus* only for the initial few hours after application, presumably while residues were still in liquid or vapor phases.

The efficiency of any pesticidal spray and the degree of control obtained is a function of the pattern of deposition of the pesticide spray droplets (Himel and Moore, 1969). In our laboratory study, *A. holoxanthus* did not react differentially to the carbaryl versus non-pesticidal deposit patterns (Chapter 4). Random contact of *A. holoxanthus* with deposits in both the carbaryl and control treatments suggested that there was no obvious attraction or repellency elicited by these deposits. For an equivalent total amount of material delivered, more numerous, yet smaller deposits elicited greater rates of parasite contact in both treated and untreated assays. The high rate of mortality of *A. holoxanthus* in the carbaryl treatment assays can be attributed to toxicity of carbaryl residues to these parasites and mortality increased as the number of drops increased. The low rates of contact in the pesticide treatments at the middle and end of the 2 hour exposure period were due to the parasites sluggish behavior. The parasites exposed to non-pesticidal deposits, on the other hand, were very active throughout the 2 hour assay period.

We successfully designed and conducted a field experiment using results of the laboratory assays to demonstrate the disruption of host-parasite relationships by high biologically toxic pesticidal treatments. The timing of repetitive spray applications was determined from the mortality data of adult *A. holoxanthus* in our bioassay (Chapter 3). The populations of FRS in the field trial began to increase noticeably in August. The application of pesticides stimulated FRS populations to increase rapidly by interfering with parasitism. Populations of FRS on citrus leaves in the carbaryl-treated plots peaked during the month of September.

We subsequently documented that parasites recovered quickly in carbaryl-treated plots, provided there were high numbers of FRS. The recovery of *A. holoxanthus* in the treated plots may be explained by the Ben-Dov and Rosen (1969) study which attributed the increased active parasitism of California red scale by *Aphytis* in Israel in pesticide-treated plots to the attraction of *Aphytis* females by the massive scale populations in the treated plots, the faster development of the parasites, the presence of a large reservoir of natural enemies on untreated trees in the immediate vicinity, and the rapid degradation of pesticide residues during these months.

In general, our results showed that the repeated use of toxic pesticides killed *A. holoxanthus and* resulted in high populations of Florida red scale on citrus leaves. The timing and interval of pesticidal application was crucial to maintaining a favorable host-parasite relationship. Pesticides toxic to beneficial parasites can be integrated into pest management strategies by considering the periodicity of pesticidal impacts on adult *A. holoxanthus* populations and by extension, to other important parasites of Florida citrus pests.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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